JOURNAL OF VIROLOGY, July 1988, p. 2234–2237
0022-538X/88/072234-04$02.00/0

Vol. 62, No. 7

Transfer of Functional Immunoglobulin G (IgG) Antibody into the Gastrointestinal Tract Accounts for IgG Clearance in Calves

THOMAS E. BESSER,[1]* TRAVIS C. McGUIRE,[2] CLIVE C. GAY,[1] AND LORI C. PRITCHETT[1]

Department of Veterinary Clinical Medicine and Surgery[1] and Department of Veterinary Microbiology and Pathology,[2] College of Veterinary Medicine, Washington State University, Pullman, Washington 99164-6610

Received 14 December 1987/Accepted 15 March 1988

The transfer of circulating immunoglobulin G1 (IgG1) antibody to the gastrointestinal tract in young calves was quantified by using bovine anti-dinitrophenol IgG1 antibody labeled with ^{125}I. The antibody was administered to newborn calves by intravenous injection, and transfer of the labeled IgG1 to the gastrointestinal tract occurred as demonstrated by excretion of protein-bound label in the feces and by the presence of the labeled IgG1 antibody in the gastrointestinal tract lumen at necropsy. Sixty-eight percent of the [^{125}I]IgG1 clearance occurred by transfer to the gastrointestinal tract. Protein-bound ^{125}I in the gastrointestinal tract lumen retained 65% of the specific dinitrophenol-binding ability of the labeled antibody originally administered. These results show that (i) transfer to the intestinal lumen is the major means of IgG1 clearance in calves, and (ii) this transfer results in antigen-binding antibody in the intestinal tract lumen. The potential contribution to enteric immunity of IgG1 reaching the intestinal lumen from circulation remains to be determined.

The mechanism by which immunoglobulin G (IgG) is cleared from circulation is not well defined in any species (32). Gastrointestinal tract organs probably account for at least some IgG clearance, since IgG has been detected in the intestinal secretions of several species (1, 6, 7, 12, 25). In cattle, detectable amounts of IgG1 reach the gastrointestinal tract lumen from the blood in clinically healthy calves and adults (25, 26), and this transfer is somewhat larger in animals with diarrhea (10, 27). However, the contribution of IgG transferred into the intestine to overall IgG clearance is not known. It is also unclear whether significant antibody activity is retained in immunoglobulin reaching the intestinal lumen.

Clearance of substantial amounts of bovine IgG1 antibody into the intestinal lumen could help explain a frequently observed relationship between higher serum passive immunoglobulin concentrations and decreased occurrence and severity of enteric disease in calves (2–4, 9–11, 14, 15, 19, 21, 29), for which no well-defined mechanism is currently known. The pathogens causing most calfhood enteric disease are noninvasive, and it is thought that for antibody to control these agents it must be present within the gastrointestinal tract lumen (13, 31). Under the hypothesis that significant amounts of circulating passive antibody enter the gastrointestinal tract lumen, the present study was undertaken with two specific objectives: (i) to quantify the clearance of circulating IgG1 into the gastrointestinal tract of neonatal calves, and (ii) to determine whether IgG1 so transferred retains antibody activity.

MATERIALS AND METHODS

DNP-specific bovine IgG1. Dinitrophenol (DNP)-binding bovine antibody was obtained as previously described (20). IgG1 was purified with anion-exchange (DE-52; Whatman, Ltd., Maidstone Kent, England) and gel-exclusion (Bio-Gel A1.5; Bio-Rad Laboratories, Richmond, Calif.) column chromatography and by absorption with protein A (protein A-Sepharose; Pharmacia Fine Chemicals, Uppsala, Sweden). Only single precipitin arcs giving a reaction of identity with bovine IgG1 (Miles Laboratories, Inc., Naperville, Ill.) were detected by Ouchterlony immunodiffusion against six different rabbit anti-whole bovine serum sera. IgG1 concentration measured by radial immunodiffusion was 1.85 mg/ml, equivalent to the protein concentration (1.72 mg/ml) estimated by optical density at 280 nm (6). IgG2 was not detectable by radial immunodiffusion (sensitivity, 0.07 mg/ml). IgA was not detectable by immunodiffusion against IgA-specific antiserum (sensitivity, 0.06 mg/ml). On sodium dodecyl sulfate-polyacrylamide gel electrophoresis with nonreducing conditions, the preparation demonstrated a single band at an M_r of 171,000; and with reducing conditions, the preparation showed two bands at positions corresponding to M_rs 27,500 and 55,900.

Radioiodination. IgG1 was labeled with ^{125}I by a chloramine T procedure (18) to a specific activity of 50,000 dpm/μg (Gamma 8000; Beckman Instruments, Fullerton, Calif.). Unbound ^{125}I was removed by anion exchange (Dowex 1X8 column; Bio-Rad), and the percentage of IgG1-bound ^{125}I was determined by trichloroacetic acid (TCA) precipitation on glass-fiber filters (16). [^{125}I]IgG1 preparations given to calves contained greater than 90% TCA-precipitable label. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography, the labeled antibody had the same number and relative intensities of bands as did the IgG1 prior to labeling.

Experimental calves. Twenty-six colostrum-deprived male Holstein calves were obtained from a commercial dairy. Twenty-four of these calves were randomly assigned to treatment groups of six calves each, differing in colostrum feeding level (1 or 3.5 liters) and age at euthanasia (5 or 10 days). The calves were fed colostrum by 3 h after birth and then were maintained on an artificial milk diet. Calves were fitted with harnesses for the collection of urine and feces and were given 2.0 ml of 20% (wt/vol) NaI in distilled water and a measured dose (45 to 110 μCi) of [^{125}I]IgG1 by intravenous injection by 48 h after birth. Two control calves were treated similarly but received [^{125}I]IgG1 per os rather than intravenously.

Excretion of [^{125}I]IgG1. Serum was sampled from each calf at 24-h intervals. After [^{125}I]IgG1 administration, fecal and

* Corresponding author.

urine output was measured and sampled at 12-h intervals. All samples were counted to determine ^{125}I activity. Net [^{125}I]IgG1 remaining in each calf on each day was determined.

Gastrointestinal tract samples. After anesthesia and exsanguination of the calves, 10-ml samples of the contents of the gall bladder, rumen, abomasum, duodenum, jejunum, and ileum were aspirated. Total contents of forestomach and abomasum, small intestine, and large intestine were then collected. Samples were collected rapidly and frozen to −70°C within 60 min of collection. Samples were later thawed and counted to determine ^{125}I concentration. TCA-precipitable ^{125}I fractions in calf secretions and serum were determined (16).

Hemoglobin concentrations were measured as described previously (17), except that each sample was buffered with 0.1 volume of 2.5 M Tris hydrochloride (pH 7.6). The modified assay was sensitive to 0.05 mg of hemoglobin per ml. Hemoglobin concentration was <0.2 mg/ml in all samples. Sample counts were corrected for ^{125}I contributed by the measured blood contamination.

Protein-bound ^{125}I in the abomasal contents, small intestinal contents, and large intestinal contents at necropsy were expressed as a percentage of net ^{125}I left in the calf. Daily excretion of [^{125}I]IgG1 into the gastrointestinal tract was estimated by multiplying the percentage of net calf ^{125}I in each organ by the number of transit times per day appropriate to that organ; eight for forestomach and small intestine and one for the large intestine (24). Because of the active recirculation of free iodide in the calf, only TCA-precipitable ^{125}I was included in these analyses (22).

DNP binding. *e*-DNP-lysine and glycine were covalently bound to CNBr-activated agarose (CNBr Sepharose; Pharmacia) to form test and control affinity matrices, respectively. Intestinal samples were clarified by centrifugation (15,000 × *g* maximum, 60 min) and filtration (Whatman no. 1 filter), and two portions of each sample, each containing 1,000 TCA-precipitable cpm, were passed over 1-ml test and control affinity columns, respectively. The columns were washed with 15 volumes of 2.0 M NaCl and 15 volumes of phosphate-buffered saline. Preliminary experiments established that elution of material bound to the affinity matrices was accomplished equally with either 8 ml of 0.1 M DNP-lysine (pH 7.4) or 3.5 ml of 0.05 M diethylamine (pH 11.5). Experimental samples were eluted with diethylamine so that eluates could be directly counted.

Experimental design and statistical analysis. The experiment was a randomized complete block design with two treatments, 12 calves per treatment, for a total of 24 calves. The effects of the amount of colostrum fed (1 or 3.5 liters) and age of the calf (5 or 10 days) on the amount of labeled IgG1 in the gastrointestinal contents at necropsy were examined. Data were analyzed by analysis of variance.

RESULTS

The clearance rate of circulating radiolabeled IgG1 was estimated from the rate of loss of [^{125}I]IgG1 from the serum of 12 calves over 8 days. The logarithm of counts per minute per milliliter of serum plotted against time stabilized to a straight line by 48 h after intravenous administration of the labeled immunoglobulin. Regression analysis of the linear portions of these plots indicated a [^{125}I]IgG1 serum half-life of 17.9 days, equivalent to daily clearance of 3.8% of a calf's IgG1 (Table 1). This half-life is consistent with previous estimates of the catabolic rate of bovine IgG1, suggesting that the labeled immunoglobulin was metabolized normally (6).

TABLE 1. Excretion of [^{125}I]IgG1 cleared from serum

Sample	^{125}I clearance[a] (%/day)	^{125}I excretion (%/day)	
		Total[b]	Protein bound[c]
Urine		2.52	0.08
Feces		1.50	1.23
Urine + feces	3.80 ± 0.34[d]	4.02 ± 0.28[d]	1.31 ± 0.22[d]

[a] Average daily percent decrease of serum ^{125}I specific activity (n = 12).
[b] Average daily percentage of net body ^{125}I in excretions (n = 24).
[c] Average daily percent of net body ^{125}I in excretions precipitable in 10% TCA (n = 24).
[d] Standard error of the mean.

^{125}I excretion in urine and feces averaged 4.02% of the calves' [^{125}I]IgG1 per day (Table 1). Therefore the ^{125}I collected from the urine and feces accounted for essentially all of the [^{125}I]IgG1 cleared from the calves. Excretion of [^{125}I]IgG1 into calf urine and feces is shown in Fig. 1. Fecal protein-bound ^{125}I averaged 1.23% of the calves' net [^{125}I]IgG1 per day, accounting for 32% of the calves' total [^{125}I]IgG1 clearance. The remainder of excreted ^{125}I (only 2.6% of which was protein bound) was collected in the urine (Table 1). Excretion rates of urinary and fecal protein-bound ^{125}I were consistent over the 10-day period (Fig. 1).

After euthanasia and sample collection at 5 or 10 days of age, the amount of labeled IgG1 in each calf's gastrointestinal tract lumen was measured; 0.14, 0.15, and 0.25% of the calves' net ^{125}I were present in the lumena of the forestomach and abomasum, small intestine, and large intestine, respectively, in the form of TCA-precipitable ^{125}I. These values correspond to daily transfer to the intestine of 2.6% of the calves' net [^{125}I]IgG1 per day, accounting for 68% of the total [^{125}I]IgG1 clearance (Table 2). The distribution of TCA-precipitable [^{125}I]IgG1 in the calves' gastrointestinal

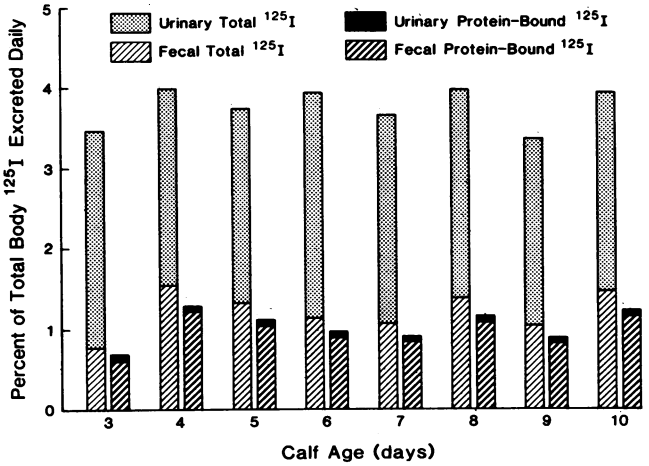

FIG. 1. Daily excretion of ^{125}I by calves after intravenous injection of [^{125}I]IgG1. For each day, the first column represents the percentage of the calves' net ^{125}I excreted that day and the second column represents that part of the first column consisting of protein-bound ^{125}I. Each column is divided to show the relative contributions of urinary and fecal ^{125}I. The daily totals of ^{125}I excretion closely approach the clearance rate of ^{125}I-IgG1 from serum (3.8% per day), and the protein-bound ^{125}I analysis shows that only fecal ^{125}I had a substantial protein-bound component.

TABLE 2. Protein-bound ^{125}I in the gastrointestinal tract of calves after intravenous administration of [^{125}I]IgG1[a]

Group	IgG1 (mg/ml) in serum	Age (days)	Luminal ^{125}I[b] (%)			FE/D[c] (%)
			RRA	SI	LI	
1	3.5	5	0.175	0.134	0.360	2.87
		10	0.147	0.150	0.103	2.48
2	16.4	5	0.083	0.142	0.289	2.01
		10	0.142	0.187	0.238	2.55

[a] Colostrum feeding level and age at necropsy did not significantly affect the fractional excretion of [^{125}I]IgG1. Twenty-four calves were tested. Group 1 calves were fed 1 liter colostrum before 3 h of age; group 2 calves were fed 3.5 liters. Mean ± standard deviation values of percentage of ^{125}I in the forestomach and abomasum, small intestine, and large intestine were 0.137 ± 0.072, 0.153 ± 0.074, 0.247 ± 0.278, and 2.57 ± 0.94%, respectively. The mean ± standard deviation calculated percentage of the total body [^{125}I]IgG1 reaching the gastrointestinal tract each day was 2.57 ± 0.94.

[b] The percentage of the total body ^{125}I-IgG1 activity present as TCA-precipitable ^{125}I in the forestomach and abomasum (RRA), small intestine (SI), and large intestine (LI) at necropsy.

[c] Fractional excretion per day (FE/D), Calculated percentage of the total body [^{125}I]IgG1 activity transferred into the gastrointestinal tract each day, on the basis of the luminal ^{125}I (%) and published values of transit time of ingesta in neonatal calves (24).

tracts is shown in Fig. 2. Calves that were 5 and 10 days old had similar fractions of total body [^{125}I]IgG1 in the gastrointestinal tract at necropsy (Table 2). The fraction of total body [^{125}I]IgG1 in the gastrointestinal tract at necropsy was not significantly affected by the volume of colostrum fed to the calf (Table 2).

The apparent discrepancy between the measured rates of clearance of [^{125}I]IgG1 to the intestine (32% excreted in the feces, 68% estimated from gastrointestinal tract contents) may be due to intestinal proteolysis of [^{125}I]IgG1 after transfer to the gastrointestinal tract. Two 48-h-old calves given oral doses of [^{125}I]IgG1 did not absorb TCA-precipitable ^{125}I to the bloodstream. Over the following days, the calves excreted 28% of the administered [^{125}I]IgG1 in their feces, predominantly as protein-bound ^{125}I, and 72% as free ^{125}I in the urine. Therefore, significant degradation of

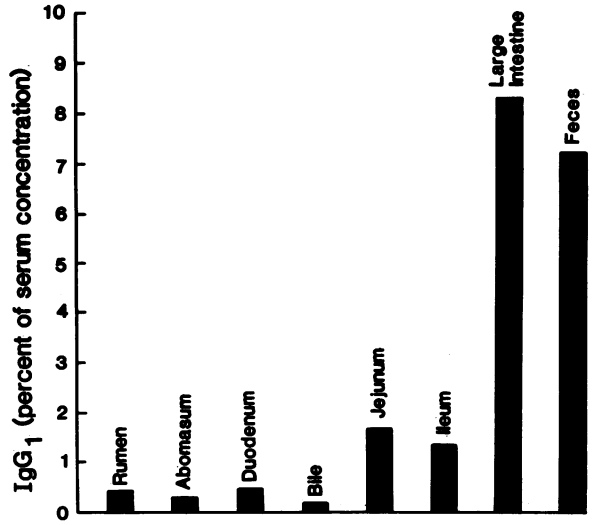

FIG. 2. Relative concentrations of protein-bound ^{125}I throughout the gastrointestinal tract after intravenous administration of [^{125}I]IgG1.

[^{125}I]IgG1 occurs in the gastrointestinal tract, and the measurement in feces and (to a lesser extent) in intestinal contents would tend to underestimate transfer of IgG1 into the gastrointestinal tract.

DNP-binding activity of the intestinal content samples was examined as one measure of functional activity of [^{125}I]IgG1 cleared into the gastrointestinal tract. TCA-precipitable ^{125}I in the calves' small and large intestinal contents specifically bound DNP approximately 67% as efficiently (66.4 ± 21.2 and 67.3 ± 20.8%, respectively) as did the labeled antibody initially administered to the calves. Binding to control antigen was not observed (0.4 ± 0.5 and 0.5 ± 0.6%, respectively).

DISCUSSION

We conclude from the data presented herein that (i) transfer to the intestine is a major clearance mechanism for circulating IgG1 in young calves and (ii) the transferred immunoglobulin retains significant functional antibody activity in the intestine. Besides further defining IgG metabolism, these results raise the possibility that circulating IgG1 cleared to the intestinal lumen is involved in intestinal immunity.

In calves given radiolabeled IgG1 intravenously, protein-bound ^{125}I was present both in feces and in the gastrointestinal tract at necropsy in amounts indicating that transfer to the gastrointestinal tract accounts for most IgG1 clearance. Unlike the present study, previous experiments to measure IgG clearance into the gastrointestinal tract have studied only small portions of the gut with the use of surgically prepared intestinal loops and/or have studied only short time periods relative to the half-life of the immunoglobulin studied (32). The results of the present study support previous work that suggested that the gastrointestinal tract is the principal clearance organ for IgG (1, 32).

Antibody reaching the gastrointestinal tract retains at least partial antibody activity. The DNP binding demonstrated in the intestinal lumen samples in this experiment shows that the protein-bound, ^{125}I-labeled material measured was labeled IgG1 or functional fragments of labeled IgG1. ^{125}I released from labeled IgG1 could conceivably bind to other proteins, although the administration of nonradioactive iodide makes the bindings of ^{125}I to other proteins unlikely (32). It is not plausible that such secondarily labeled proteins would specifically bind DNP.

The DNP-binding activity demonstrates that a functional role for circulating IgG1 cleared into the gastrointestinal tract is possible and suggests that clearance of functional circulating IgG1 antibody into the gastrointestinal tract may enhance other intestinal immune mechanisms. This suggestion could help to explain the correlations that have been observed between the passive serum immunoglobulin concentrations of neonatal calves and their resistance to morbidity and mortality associated with diarrheal disease in the first weeks of life. The transfer of circulating IgG1 to the gastrointestinal tract lumen indicates that a calf that absorbs 100 g of IgG1 from colostrum would subsequently secrete 1 to 4 g back into the gut each day during the first 2 weeks of life. The clearance of labeled IgG1 to the intestine was not inhibited in calves with high serum IgG1 concentrations (see above), so calves with higher serum IgG1 concentrations would be expected to clear larger amounts of IgG1 to the gastrointestinal tract lumen.

Only calves in the first 10 days of life were studied in this experiment, so no conclusions can be drawn regarding the

clearance of circulating IgG1 via the intestine in older cattle. However, the appearance of at least some blood IgG1 in the intestinal lumen of adult cattle and the similar clearance rates of circulating IgG1 in adult cattle (5, 28) and in the calves in this experiment suggest that intestinal clearance of IgG1 is of similar magnitude in adult cattle.

Other workers (25) have demonstrated that IgG1 is the predominant immunoglobulin in the intestinal lumen of young calves, with other immunoglobulins appearing in intestinal secretions by 4 weeks of age, and adult immunoglobulin ratios not attained until 10 to 12 weeks of age. A significant fraction of intestinal IgG1 in young calves originated in serum, resulting in a positive correlation between the percentage of intestinal serum-derived IgG1 and the serum IgG1 concentration. IgG1 decreased from 98 to 48% of total intestinal immunoglobulin content in calves aged 2 and 14 weeks, respectively (23). The results of the present study supports the findings of these investigators regarding the appearance of circulating IgG1 in the intestinal lumen. In contrast, others found IgM to be the predominant immunoglobulin in intestinal loops in preruminant calves, with only minor amounts of IgG present (30). This apparently conflicting observation could be reconciled if the calves studied (30) had relatively low passive immunoglobulin concentrations, resulting in a low rate of transfer of IgG1 into intestine.

This study provides no information regarding the mechanism of IgG1 appearance in the gastrointestinal tract. Conflicting results of other studies have provided evidence both for and against a selective transport of IgG1 from the blood to the gastrointestinal tract (7, 8). However, regardless of the mechanism of transfer, the clearance of gram quantities of IgG retaining at least some antibody function into the gastrointestinal tract each day is worthy of further investigation for a functional role in intestinal immunity in the bovine species.

ACKNOWLEDGMENTS

This work was supported by the Washington State Dairy Products Commission, by Washington State University Agricultural Research Committee grant 10A-3073-0602, and by U.S. Department of Agriculture grant 86-CRCR-1-2169.

LITERATURE CITED

1. **Andersen, S. B., J. Glenert, and K. Wallevik.** 1963. Gamma globulin turnover and intestinal degradation of gamma globulin in the dog. J. Clin. Invest. **42**:1873–1881.
2. **Blom, J. Y.** 1982. The relationship between serum immunoglobulin levels and incidence of respiratory disease and enteritis in calves. Nord. Vet. Med. **34**:276–281.
3. **Boyd, J. W.** 1972. The relationship between serum immune globulin deficiency and disease in calves: a farm survey. Vet. Rec. **90**:645–649.
4. **Boyd, J. W., J. R. Baker, and A. Leyland.** 1974. Neonatal diarrhea in calves. Vet. Rec. **95**:310–313.
5. **Brandon, M. R., D. L. Watson, and A. K. Lascelles.** 1971. The mechanism of transfer of immunoglobulins into mammary secretions of cows. Aust. J. Exp. Biol. Med. Sci. **49**:613–623.
6. **Butler, J. E.** 1983. Bovine immunoglobulins: an augmented review. Vet. Immunol. Immunopathol. **4**:43–152.
7. **Cripps, A. W., A. J. Husband, and A. K. Lascelles.** 1974. The origin of immunoglobulins in intestinal secretions of sheep. Aust. J. Exp. Biol. Med. Sci. **52**:711–716.
8. **Curtain, C. C., B. L. Clark, and J. H. Duffy.** 1971. The origins of the immunoglobulins in the mucus secretions in cattle. Clin. Exp. Immunol. **8**:335–344.
9. **Fisher, E. W., and G. H. de la Fuente.** 1971. Antibiotics and calf diarrhea—the effect of serum immune globulin concentration. Vet. Rec. **89**:579–582.
10. **Fisher, E. W., A. A. Martinez, Z. Trainin, and R. Meirom.** 1975. Studies of neonatal calf diarrhea. II. Serum and faecal immune globulins in enteric colibacillosis. Br. Vet. J. **131**:402–414.
11. **Frerking, H., and T. Aeikens.** 1978. About the importance of colostrum for the newborn calf. Ann. Rech. Vet. **9**:361–365.
12. **Fubara, E. S., and R. Freter.** 1972. Availability of locally synthesized and systemic antibodies in the intestine. Infect. Immun. **6**:965–981.
13. **Gay, C. C.** 1983. Failure of passive transfer of colostral immunoglobulins and neonatal disease in calves: a review, p. 346–364. *In* Proceedings of the Fourth International Symposium on Neonatal Diarrhea. Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada.
14. **Gay, C. C., N. Anderson, E. W. Fisher, and A. D. McEwan.** 1965. Gamma globulin levels and neonatal mortality in market calves. Vet. Rec. **77**:148.
15. **Hurvell, B., and H. Fey.** 1970. Comparative studies on the gammaglobulin levels in sera of market calves in relation to their health. Acta Vet. Scand. **11**:341–360.
16. **Johnstone, A., and R. Thorpe.** 1982. Immunochemistry in practice. Blackwell Scientific Publications, Oxford.
17. **Lijana, R. C., and M. C. Williams.** 1979. Tetramethylbenzidine—a substitute for benzidine in hemoglobin analysis. J. Lab. Clin. Med. **94**:266–276.
18. **McConahey, P. J., and F. J. Dixon.** 1966. A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy **29**:185–189.
19. **McEwan, A. D., E. W. Fisher, and I. E. Selman.** 1970. Observations on the immune globulin levels of neonatal calves and their relationship to disease. J. Comp. Pathol. **80**:259.
20. **McGuire, T. C., A. J. Musoke, and T. Kurtti.** 1979. Functional properties of bovine IgG_1 and IgG_2: interaction with complement, macrophages, neutrophils and skin. Immunology **39**: 249–256.
21. **McNulty, M. S., J. B. McFerran, D. G. Bryson, E. F. Logan, and W. L. Curran.** 1976. Studies on rotavirus infection and diarrhea in young calves. Vet. Rec. **99**:228.
22. **Miller, J. K., P. T. Chandler, and R. G. Cragle.** 1971. Radioiodine absorption and secretion by the calf abomasum. J. Dairy Sci. **54**:397–400.
23. **Morgan, K. L., F. J. Bourne, T. J. Newby, and P. A. Bradley.** 1981. Humoral factors in the secretory immune system of ruminants. Adv. Exp. Med. Biol. **137**:391–412.
24. **Mylrea, P. J.** 1966. Digestion of milk in young calves. Res. Vet. Sci. **7**:333–341.
25. **Newby, T. J., and F. J. Bourne.** 1976. The nature of the local immune system of the bovine small intestine. Immunology **31**:475–480.
26. **Nielsen, K., and J. Dich.** 1965. Passage of ^{131}I-albumin and ^{125}I-gamma globulin into the small intestine of calves. Acta Vet. Scand. **6**:249–260.
27. **Nielsen, K., and P. Nansen.** 1967. Metabolism of bovine immunoglobulin. II. Metabolism of bovine IgG in cattle with secondary hypoimmunoglobulinemia. Can. J. Comp. Med. Vet. Sci. **31**:106–110.
28. **Nielsen, K., J. Sheppard, W. Holmes, and I. Tizard.** 1978. Experimental bovine trypanosomiasis. Changes in the catabolism of serum immunoglobulins and complement components in infected cattle. Immunology **35**:811–816.
29. **Penhale, W. J., G. Christie, A. D. McEwan, E. W. Fisher, and I. E. Selman.** 1970. Quantitative studies on bovine immunoglobulins. II. Plasma Ig levels in market calves and their relationship to neonatal infection. Br. Vet. J. **126**:30–37.
30. **Porter, P., D. E. Noakes, and W. D. Allen.** 1972. Intestinal secretion of immunoglobulins in the preruminant calf. Immunology **23**:299–312.
31. **Tzipori, S.** 1981. The aetiology and diagnosis of calf diarrhea. Vet. Rec. **108**:510–514.
32. **Waldmann, T. A., and W. Strober.** 1969. Metabolism of immunoglobulins. Prog. Allergy **13**:1–110.